

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is used for the qualitative and quantitative analysis of components in mixtures of organic materials. It is particularly useful for the analysis of organic compounds with low volatility or that degrade when heated.

Principle of Technique

Samples are injected through an injection port into a flowing liquid eluant and are then transported onto the front of a chromatographic column. The column is packed with a stationary phase, such as silica gel, alumina, C-18, or C-8 bonded packings. As eluant is pumped through the column, separation is achieved by the interaction of the analytes with the column packing and the eluant. As the eluant and analytes pass through the detector, a signal is produced. Detection is usually based on ultraviolet absorption.

Samples

Form. Liquids or solids.

Size. The amount of sample for analysis may vary from a few milligrams to the several hundred grams required for soil extractions.

Preparation. Liquid samples can often be analyzed as received; solid samples require a prior extraction.

Limitations

Analytes must be freely soluble or miscible in the HPLC eluant. Typical eluants for reverse-phase HPLC are methanol, tetrahydrofuran, and acetonitrile in water. Hexane, dichloromethane, and chloroform are used commonly for normal phase HPLC.

It is difficult to make unambiguous identification of a particular component, especially at trace levels. On-

line ultraviolet spectroscopic measurements are possible during analysis for major components, and can be compared with reference spectra for identification. Subsequent analysis by infrared spectroscopy or mass spectroscopy may be necessary in some cases.

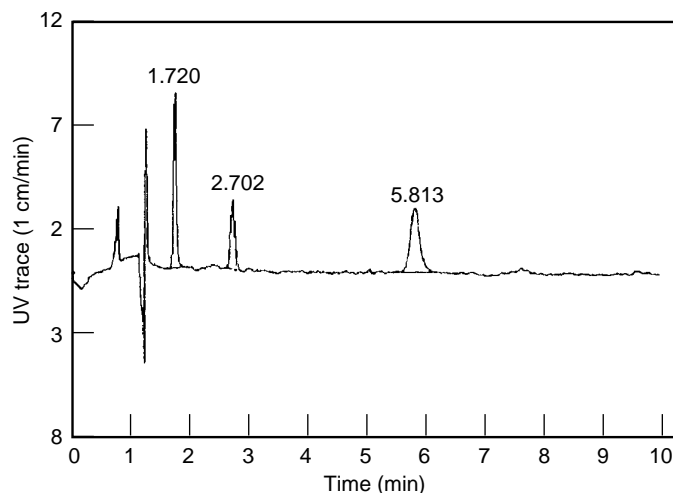
Not all materials are possible candidates for HPLC analysis because of solubility and detection limitations. Because the packing and eluant used to separate the analytes are specific for a certain type of analysis, HPLC is not a screening technique. The analytes of interest must be known, and reference materials are used to obtain the desired data.

Estimated Analysis Time

Calibration is required for all concentration measurements because of the selective nature of the detectors (UV or refractive index) and the

Examples of Applications

- Analysis of explosives, thermally unstable materials, polymers, and epoxy resins.
- Determination of trace amounts (<100 ppm) of explosives in soil or water.



UV detector trace from HPLC analysis of a standard of HMX (1.720 min), RDX (2.702 min), and TNT (5.813 min). Concentration was 320 µg/L (320 ppb).

response factors of the analytes. Instrument time required for a separation can vary from 30 min to 2 h, provided a standard method is in place to perform the required separation. When no method has been previously published for materials submitted for analysis, method development is required.

Capabilities of Related Techniques

Ultraviolet spectrophotometric analysis can be used to determine detector response of specific reference materials. Gas chromatography (GC) analysis may be possible for thermally stable materials.

The various techniques in which gas chromatography or HPLC are coupled to mass spectrometry may be used for further identification of unknown species.